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Molecular and Cellular Basis of the Mammary Gland Susceptibility to Carcinogenesis

by Jose Russo,* Lee K. Tay,* Daniel R. Ciocca* and Irma H. Russo*

Mammary carcinomas induced by the administration of 7,12-dimethylbenz(a)anthracene (DMBA) to young virgin rats arise from undifferentiated terminal ductal structures called terminal end buds (TEBs). TEBs that normally differentiate into alveolar buds (ABs) and lobules under the influence of DMBA develop intraductal proliferations which progress to carcinoma. The high susceptibility of the young virgin rat TEBs to neoplastic transformation is due to its large proliferative compartment, with cells cycling every 10 hr, and to a higher ^{3}H-DMBA uptake. Progressive differentiation of TEBs into ABs and lobules or their regression to terminal ducts (TDs) is seen with aging. Complete differentiation of the gland is attained only through pregnancy and lactation. The greater differentiation of the gland is manifested as permanent structural changes, consisting in the disappearance of TEBs and in a diminution of the number of TDs due to their differentiation into ABs and lobules. This greater differentiation results in a diminished or total refractoriness of the gland to the carcinogen because ABs and lobules have a lower proliferative compartment and a longer cell cycle than TEBs and TDs. Cells of parous rats have both *in vivo* and *in vitro* a lower DMBA-DNA binding capacity, a lower DNA synthesis and a greater ability to repair DMBA damaged DNA than cells of young virgin rats. The more efficient DNA repair capacity of the parous rat mammary gland is demonstrated by the induction of unscheduled DNA synthesis and a removal of DMBA-DNA adducts.

Introduction

Breast cancer is a tumor that arises from a hyperplastic growth of the mammary gland epithelium (*1-6*). Although the etiologic agent(s) which stimulate the epithelial growth that eventually evolves into neoplasia is not yet known, certain common denominators have been found (Table 1) (*7-75*). Epidemiologic observations reveal that mammary carcinomas occur more frequently in nulliparous than in multiparous women (*27-29, 47, 76-78*), in women having an early menarche (*12-19, 79, 80*) and in those whose first pregnancy occurs after 25 years of age (*12-15, 81*). Pregnancy before age 18 (*11, 12, 81, 82*) and late menarche (*79*) are related to a lower risk of breast carcinoma. Just how women are protected by early pregnancy has been widely discussed, but there is very little information beyond the fact itself. The understanding of the mechanism by which early pregnancy protects women from breast cancer requires a thorough comprehension of the pathogenesis of the disease and how the reproductive history influences the susceptibility of the mammary gland to carcinogenesis. Due to sampling and ethical limitations intrinsic to human experimentation, what is needed is an experimental model for breast tumors which mimics the most significant aspects of the human disease. Recent studies (*62, 83-86*) suggest that

Table 1. Common denominators in the natural history of breast carcinoma.

Endocrine or hormone-related factors
Age at menarche, first pregnancy, and menopause
Parity
Androgen and estrogen secretion
Anovulatory cycles
Environmental factors
Geography
Diet
Socioeconomic status
Familial and/or heredity factors
Familial aggregation
Specific antigens
Cerumen type

*Department of Pathology, Michigan Cancer Foundation, 110 E. Warren Avenue, Detroit, MI 48201.

mammary carcinomas induced in Sprague-Dawley rats by 7,12-dimethylbenz(a)anthracene (DMBA) constitutes such a model. DMBA-induced rat mammary carcinomas are hormone-dependent adenocarcinomas histologically similar to human breast tumors (*62, 87,88*). Tumor incidence is higher when the carcinogen is administered to nulliparous rats (*62, 83, 89*). Although tumor growth is stimulated by pregnancy (*88, 90*), a full-term pregnancy and lactation prior to carcinogen administration inhibits tumor development (*83, 85*). Therefore, the relationship between nulliparity and the probability of the female rat of developing mammary cancer after carcinogen administration is similar to that observed in human females.

Other carcinogens such as *N*-methyl-*N*-nitrosourea (*91*) and aromatic amine derivatives have also been used to induce mammary carcinomas (*92, 93*). However, the model system most used for the study of the pathogenesis of the disease as well as the understanding of the mechanism of susceptibility is the DMBA-induced mammary carcinoma rat experimental model. It is likely that the knowledge gained with this experimental model could also be applied to mammary cancers induced by other carcinogens and ultimately for the understanding of the human disease.

Induction of Mammary Tumors by Polycyclic Aromatic Hydrocarbons (PAH)

Many PAHs are potent carcinogens and mutagens that are widely distributed as pollutants in the environment. Of great significance in breast carcinogenesis is the finding of Huggins and co-workers (*94, 95*) that under optimal conditions, intragastric instillation of DMBA or benzo(a)pyrene [B(a)P]) to rats induces mammary cancers. Mammary cancers have also been detected when PAH are administered by other means (*96, 97*); they have also been regarded

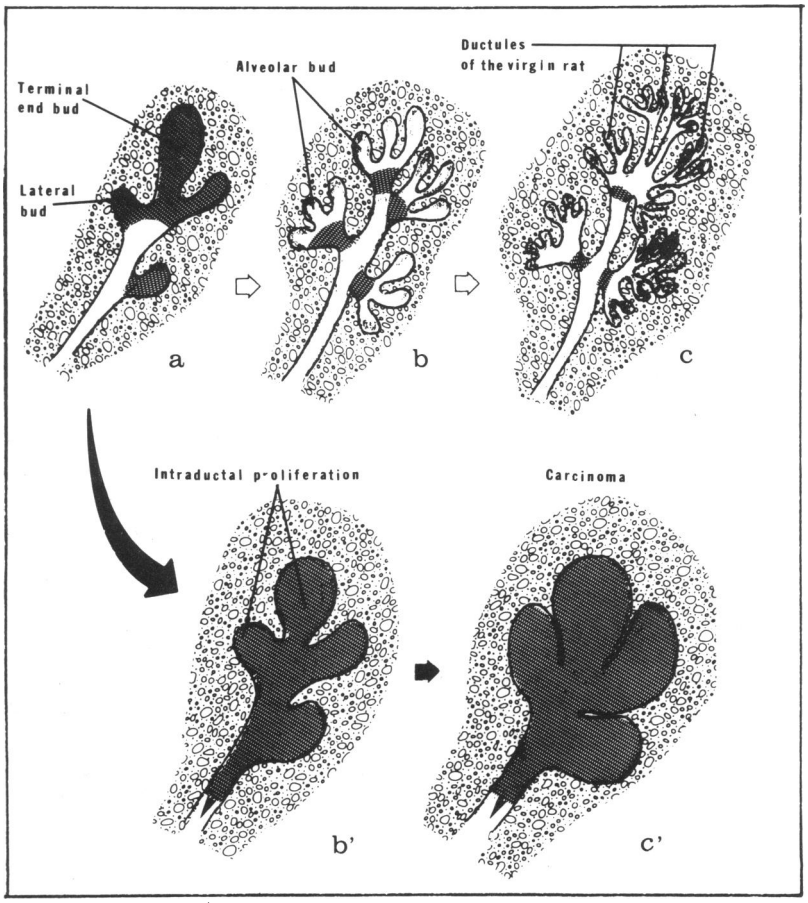

FIGURE 1. Postnatal development of the rat mammary gland and site of origin of DMBA-induced mammary carcinoma: (*a*) terminal end buds at 50 days of age; (*b*) TEBs differentiated into alveolar buds; (*c*) alveolar buds differentiated in the small ductules of the virginal lobules; (*b'*) intraductal proliferation originated from TEB after DMBA treatment; (*c'*) microtumor.

as direct-acting carcinogens because microgram quantities are capable of causing cancer at the site of administration (*98*).

Pathogenesis of Rat Mammary Carcinomas

DMBA administration to young virgin rats induces a high incidence of carcinomas. This is due to the fact that the mammary gland of young virgin animals is in an early stage of development and is composed of numerous terminal end buds (TEBs) which are actively differentiating into alveolar buds (ABs) (Fig. 1). TEBs (Fig. 1*a*) present in the mammary gland at the time of carcinogen administration are transformed by DMBA, becoming larger due to intraductal proliferation (IDP) of the lining epithelium (Fig. 1*b* and Fig. 2). IDPs increase progressively in size and become confluent, leading to the formation of microtumors (Fig. 1*c*) which histologically are adenocarcinomas (*62, 87, 99, 100*). Although the differentiation of TEBs into ABs is inhibited by DMBA treatment, not all the TEBs present in the mammary gland at the time of DMBA administration progress to IDPs. Some of them differentiate into ABs and occasional lobular development is observed. Those TEBs that are already differentiated into ABs prior to DMBA administration do not develop carcinomas. Most of them either remain unmodified or proliferate moderately, forming microscopic adenomas, or undergo dilatation of the lumen, giving rise to hyperplastic alveolar nodules (HAN) and cysts (Fig. 3). These observations indicate that the carcinogen alters the normal process of differentiation of the gland. The carcinogen requires an adequate

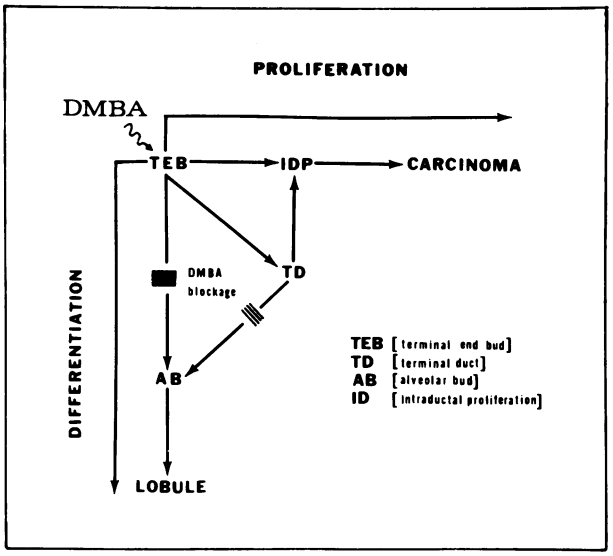

FIGURE 2. Schematic representation of the effects of DMBA on cellular differentiation and proliferation. TEB, terminal end bud; TD, terminal duct; AB, alveolar bud; ID, intraductal proliferation.

structural target that determines the type of lesion induced depending upon the area of the mammary gland with which it is in contact. Thus, the more differentiated the structure at the time of carcinogen administration, the more benign and organized is the lesion which develops (Fig. 3) (*101, 102*).

Cell of Origin of Mammary Carcinoma

The mammary gland epithelium is composed of three main cell types, intermediate, dark and myo-

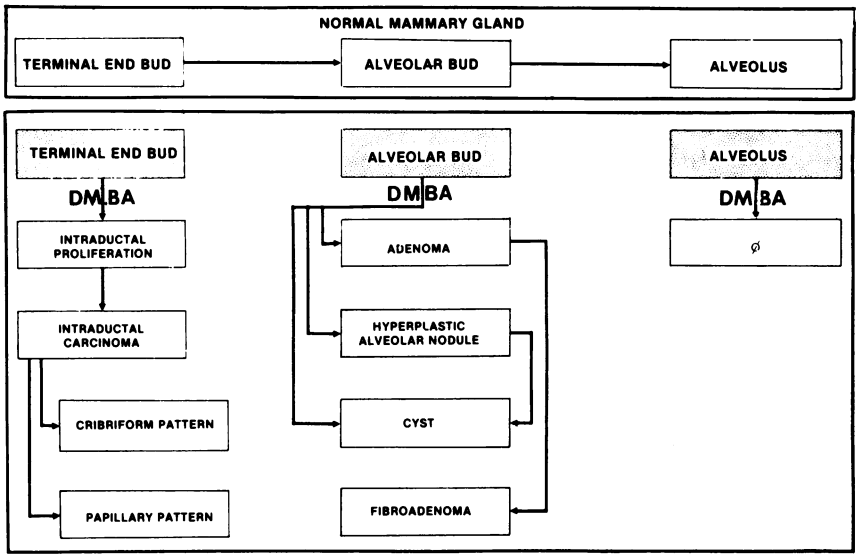

FIGURE 3. Evolution of the lesions induced by DMBA in the TEB and AB of the rat mammary gland.

epithelial cells, which possess morphological and cell kinetic properties that allow the differentiation of one cell type from another (*103, 104*). These cells are present in a constant proportion in the various compartments, namely TEB, TD, AB and lobules of the gland (Fig. 4). Following DMBA administration, the distribution of dark and intermediate cells changes in the TD and TEB, with a significant increase in the number of intermediate cells at the expense of dark cells. Myoepithelial cells (MC) are unaffected. The proportion of intermediate cells continues to increase with tumor age and in the well-developed tumor they represent nearly 90% of the total number of cells, while dark cells are reduced to approximately 10%, and MC become undistinguishable (Fig. 4). The intermediate cell type seems to be the site of neoplastic proliferation, as suggested by its higher DNA-LI after DMBA administration, which has been shown to depress the DNA-LI of other cell types, resulting in a progressive increase of intermediate cells during the carcinogenetic process.

Mammary Gland Differentiation as a Determinant of Susceptibility to Carcinogenesis

Differentiation is considered to be the process by which a cell or a structure advances from an immature to a mature or specialized state. In the case of the rat mammary gland, the sequence TEB→AB→ lobules, marks the process of differentiation. The

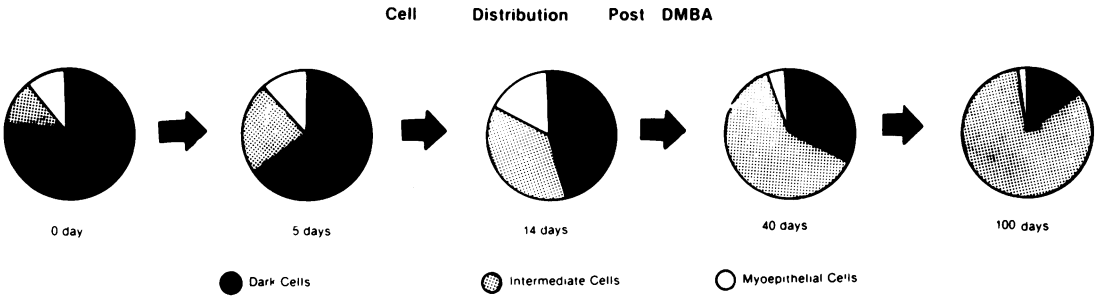

FIGURE 4. Effect of DMBA on the distribution of cell population of the TEB and TD of mammary glands. Adapted from Russo, Tait, and Russo (*105*).

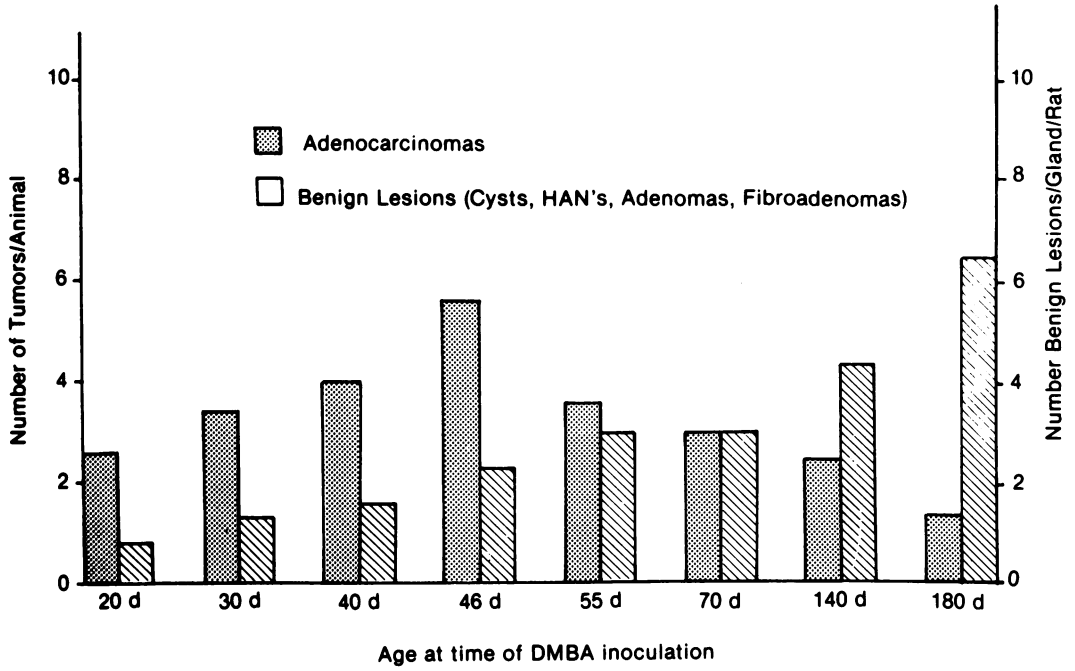

FIGURE 5. Incidence of adenocarcinomas and benign lesions in Sprague-Dawley rats treated at different ages with DMBA. Adapted from Russo, Wilgus and Russo (*102*) with permission of the editor.

highest density of TEBs (TEBs/mm^2) is found in the mammary gland of 21-day-old rats and decreases steadily until the rats reach 63 days of age (*101, 102*). This decrease is accompanied by the differentiation of this structure into ABs and of these into lobules. The administration of DMBA to rats of different ages induces tumors with an incidence which is directly proportional to the density of TEBs which are ready to differentiate into ABs. The highest incidence of carcinomas and number of tumors per animal is obtained when DMBA is administered to rats which are 40 and 46 days old, a period when TEBs are most actively differentiating into ABs (Fig. 5).

The susceptibility of the mammary gland to carcinogenesis decreases significantly with age (*102, 106*). This has been explained as a consequence of a diminution in the number of undifferentiated structures. The sharp decrease in TEBs/mm^2 observed in animals older than 55 days is also accompanied by a lower incidence of tumor formation as well as a lower number of tumors per animal with increasing age (*101, 102*). ABs which are more differentiated structures are the site of origin of benign lesions, the number of which increases with aging as a consequence of the greater density of ABs present in the gland when DMBA is administered at older ages (Fig. 5) (*101, 102*).

Mammary tumorigenesis by carcinogens is also inhibited in rats in which mammary growth has been prestimulated by hypothalamic lesions (*107*), or pituitary grafting (*108*) or when the carcinogen is administered to lactating rats (*89*). Decreased tumor incidence has also been observed when mice (*109*) and rats (*90, 106, 110*) are inoculated with chemical carcinogens after pregnancy and lactation. The protective effect of pregnancy and lactation that extends to the postweaning period has been attributed to the higher degree of differentiation of the mammary gland (*101*). This protective effect is manifested by a significantly low incidence of carcinomas developed by parous rats when a single dose of DMBA is administered after the glands have regressed to a resting stage (Table 2). This indicates that it is not the hormonal status of pregnancy and lactation that protect the gland, but the permanent changes induced in the gland structure and in the biological properties of the gland epithelium which are independent of the hormonal status of the host (*111*). In order to be protective pregnancy must be completed. Pregnancy interruption slightly increases the incidence of carcinomas, and this is related to an incomplete differentiation of the gland (Fig. 6) (*112*).

Susceptibility of the Mammary Gland to Carcinogenesis as a Function of Differentiation and Cell Kinetics

The degree of differentiation of the mammary gland can be described at a given time in the life span of the rat according to the number of TEBs, TDs, ABs and lobules. A decrease in the density of TEBs is observed with aging, and in the glands of parous rats, TEBs are undetectable (*101, 102, 106*). The high susceptibility of TEBs to carcinogenesis is the result of certain characteristics of its epithelium, mainly a high proliferative activity, reflected

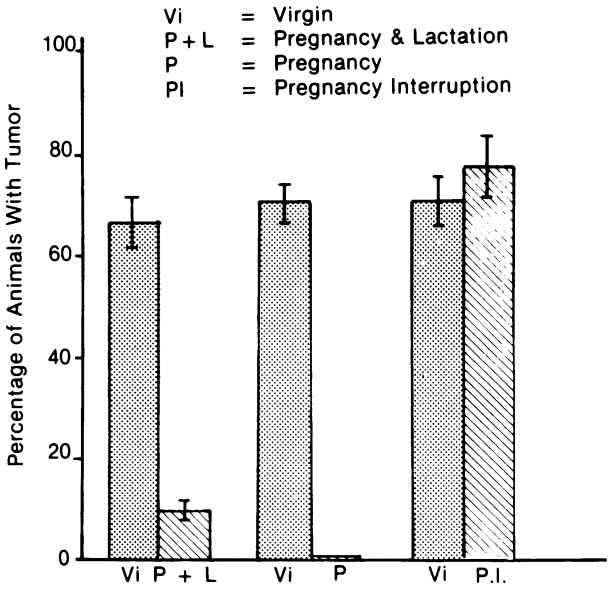

FIGURE 6. Incidence of mammary tumors (adenocarcinomas) in the mammary gland of DMBA-treated rats. Adapted from Russo and Russo (*112*) with permission of the editor.

Table 2. Tumor incidence and tumor types developed in rat mammary glands after DMBA administration.[a]

Group	No. of rats with tumor/ no. of rats	Percent of rats with tumor	Total no. of tumors	Carcinomas		Fibroadenomas		Latency period, wk.
				No.	%	No.	%	
Young virgin	16/21	76.2	34	34	100	0	0	8-9
Old virgin	14/26	53.8	19	12	63	7	36	11-21
Multiparous	18/51	35.3	19	4	21	15	79	13-19

[a]Data from Russo and Russo (*106*).

in high mitotic and DNA synthetic indices (Table 3) (*106*). The influence of these factors has been substantiated by determining the length of each one of the phases of the cell cycle (T_c) and the growth fraction (GF) of the epithelium lining the various terminal structures of the mammary gland (*113*).

The TEB possesses the highest mitotic and DNA synthetic activities and GF (*113*), and its epithelium has a very short T_c (Fig.7). The progressive differentiation of TEB to AB and lobule is accompanied by a diminution in mitotic and DNA synthetic activities and GF, while T_c lengthens, mainly due to lengthening of the G_1 phase (Fig. 7). These changes resulting from differentiation are observed within a single gland, but they are accentuated with aging (Fig. 7).

Complete differentiation of the mammary gland as a consequence of pregnancy and lactation eliminates the undifferentiated structures, resulting in a concomitant decrease in mitotic and DNA labeling indices, GF and further lengthening of T_c (*113*). No variations in the length of S phase are observed either with aging or parity or for the different compartments of the gland. Pregnancy, therefore, induces two basic changes in cell kinetic parameters in the mammary gland: one is the increase in the size of the nonproliferative compartment (G_o), and the second is the lengthening of G_1 phase of the cell cycle (Fig. 7) (*113*). These cell kinetic changes could explain the refractoriness of the mammary gland to DMBA-induced carcinogenesis. That is, due to the

Table 3. Percentage of TEB, TD and AB-containing labeled cells and percentage of labeled cells in these structures.[a]

Group	TEB		TD		AB	
	% labeled cells[b]	% labeled TEB	% labeled cells[b]	% labeled TD	% labeled cells[b]	% labeled AB
Young virgin	34.4 ± 7.6	100	12.3 ± 5.8	70	7.9 ± 3.3	50
Old virgin	14.8 ± 4.7	100	4.9 ± 3.4	28	10.9 ± 1.7	5
Multiparous	0.0	0	0.3 ± 0.5	8.2	0.3 ± 0.05	0.9

[a]Data from Russo and Russo (*106*).
[b]Values are means ± SD of the DNA labeling indexes at the moment of DMBA administration.

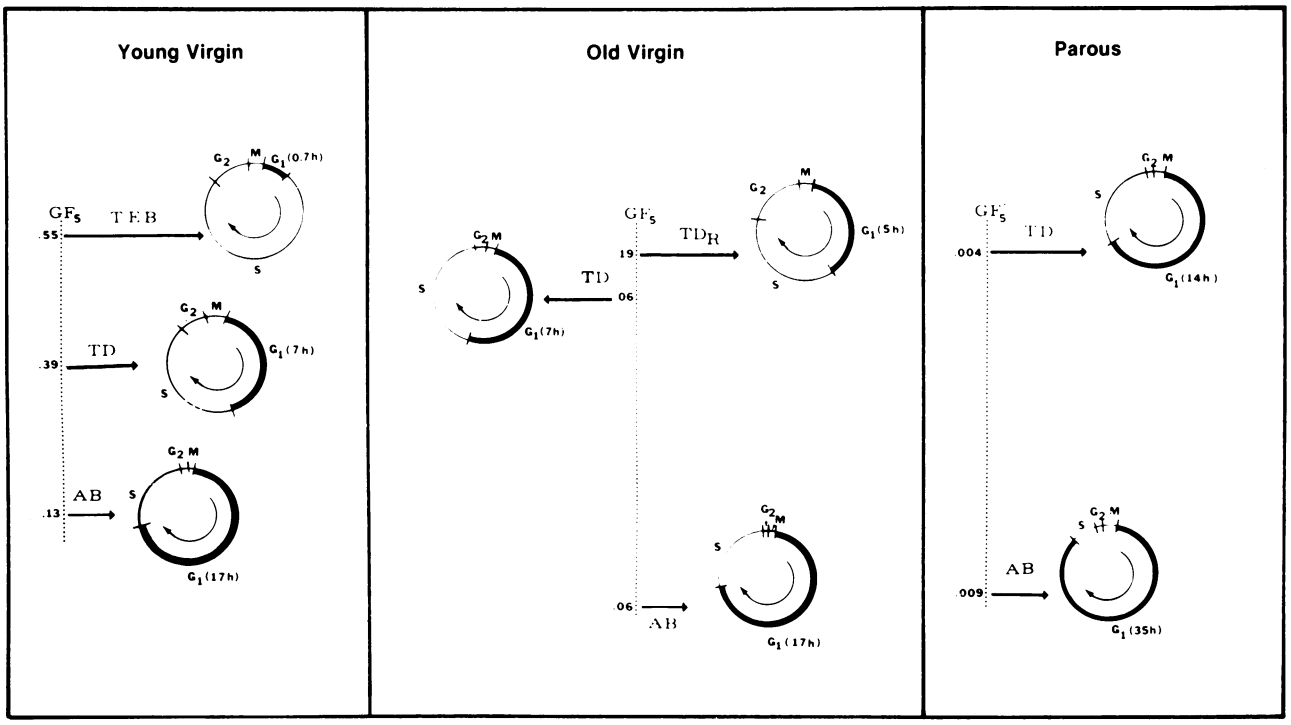

FIGURE 7. Schematic representations of the cell kinetics parameters in the mammary gland of young, old virgin and parous rats. TEB, terminal end buds; TD, terminal ducts, AB, alveolar buds; GF_5, growth fraction expressed as the total number of labeled nuclei/100 cells after 5 days of continuous infusion of [^{3}H]dThd. The diagram of the cell cycle represents the relative length of the various phases of the cycle for each of the structures shown. Adapted from Russo and Russo (*113*) with permission from the editor.

effect of pregnancy on differentiation of the mammary gland, TEBs and TDs shift all their cells to the formation of ABs and lobules. When the lobular structures regress from their functional activity, the cells present in the postpregnancy and/or postlactational state have entered G_0 or a quiescent state. If the cells are treated at this point *in vivo* with a carcinogen, they are refractory to carcinoma development (*106*) and *in vitro* are less susceptible to the effects of DMBA (*114, 115*).

Endocrinological Milieu and Susceptibility of the Rat Mammary Gland to Carcinogenesis

Since the mammary gland is under hormonal control (*116*) and the reproductive history affects the risk of mammary cancer, interest has been generated toward the study of the endocrinological status of rats with different susceptibility to carcinogenesis. Previous reports have shown variations in hormonal levels in different strains of rats and mice with varying incidence of mammary tumors (*117-120*). However, when rats of the same strain but with different susceptibility to carcinogenesis because of variations in age and in reproductive history are compared, it is found that such differences in susceptibility are not related to prolactin (PRL) or estrogen levels at the time of, or after DMBA administration (Figs. 8-10) (*121, 122*). Several changes have been observed in young virgin (YV), old virgin (OV) and parous (P) rats after DMBA administration; the most conspicuous are: hyperplasia of pituitary PRL cells, high serum PRL levels, nodular hyperplasia of the adrenal cortex, high serum estradiol levels, and lack of adrenal necrosis in all parous rats and in some old virgin rats.

Notwithstanding the modifications in the hormonal milieu observed in susceptible and nonsusceptible rats after DMBA administration, there is not a clear correlation between the hormonal changes induced and the degree of susceptibility of the mammary gland to carcinogenesis, since parous rats that received the inducer (DMBA) and have the same endocrinological milieu as young and old virgin rats do not develop tumors. This supports the importance of the degree of the mammary gland differentiation at the moment of carcinogen administration as a main factor in the different susceptibility of the gland to carcinogenesis (*101, 113*).

In Vitro Study of Mammary Gland Susceptibility to Carcinogenesis

The methods used for culture and the characterization and identification of mammary epithelial cells

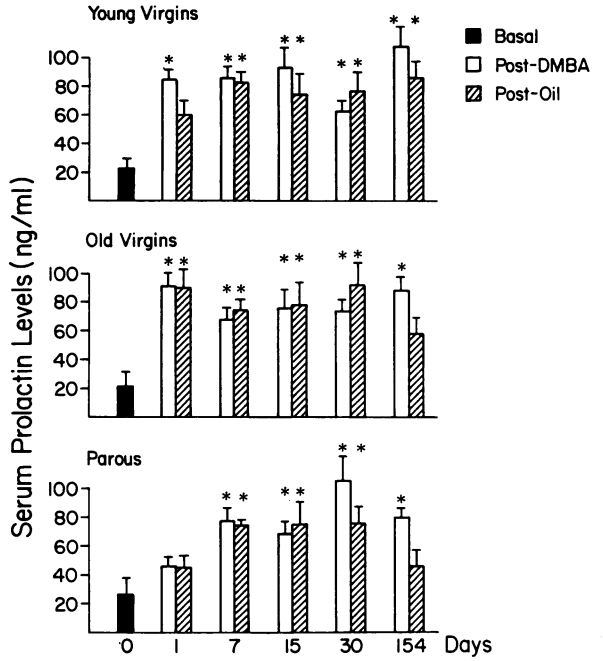

FIGURE 8. Serum PRL levels (RIA) in YV, OV and P rats before (basal) and after DMBA or oil administration. The values were compared with basal levels; statistically significant differences (*) were considered with $p < 0.005$.

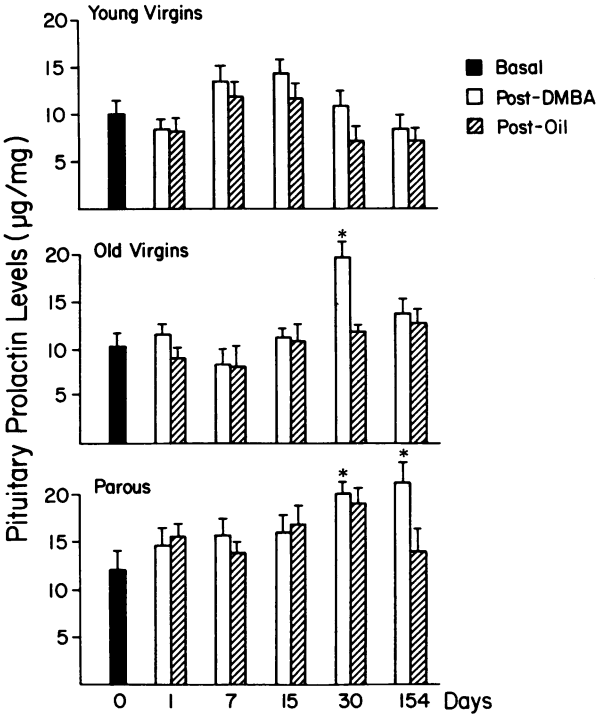

FIGURE 9. Pituitary PRL levels (RIA) in YV, OV and P rats before (basal) and after DMBA or oil administration. The values were compared with basal levels; statistically significant differences (*) were considered with $p < 0.005$.

have been described (*115, 123, 124*). Differences in the growth pattern of mammary gland epithelial cells *in vitro* correlate with the observed cell kinetic variations related to the degree of differentiation of the gland. Epithelial cells from the mammary gland of young virgin rats adapt to the culture conditions rapidly, acting as if the cells were in the logarithmic phase of growth prior to plating. The cells from old virgin and parous rats, on the other hand, require a certain time during which the proliferating cells adapt to the culture condition, as evidenced by the presence of a lag phase of cell growth (Table 4). The number of proliferating cells decreases with age and parity, as evidenced by peak DNA synthetic activity. This means that even when normal growth restraints are removed as the cells are cultured *in vitro*, only certain cells in the population are able to proliferate, which implies that the differences are intrinsic to the epithelial cells and not to host factors (*114*).

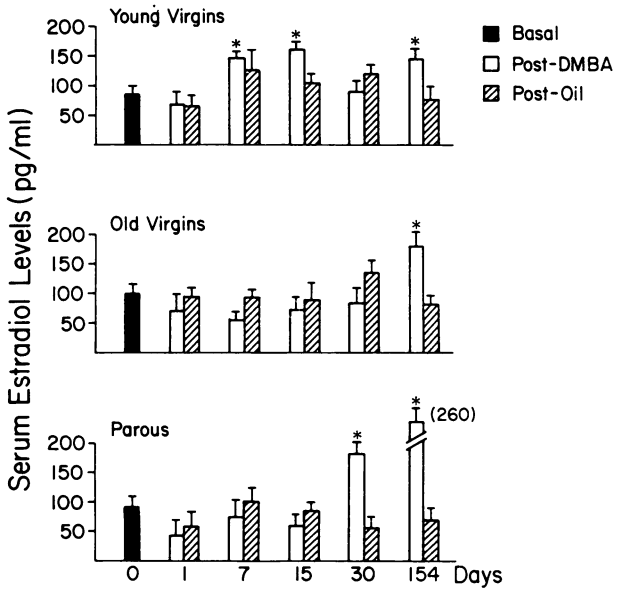

FIGURE 10. Serum estradiol levels (RIA) in YV, OV and P rats before (basal) and after DMBA or oil administration. The values were compared with basal levels; significant differences (*) were considered with $p < 0.005$.

An important difference in the growth curve of young virgin, old virgin and parous rat mammary cells is that the lag phase is lengthened in the two latter groups, which correlates with observations *in vivo* (*113, 126*). This initial resting state observed in old virgin and parous rat mammary gland primary cultures results in a lower DMBA-DNA binding (*115*) and acts as a protective mechanism against the toxic effect of DMBA when the carcinogen is added immediately after plating, which is more pronounced on the cells of young virgin rats (*123, 124*). DMBA added to cells at the peak of DNA synthesis induces a greater growth inhibition at lower doses than when the carcinogen is added during the first phase of growth. These observations confirm that cultured cells are generally more sensitive if the carcinogen is added at a time when the cells are actively growing and synthesizing DNA (*126-132*).

Metabolic Activation of DMBA

In common with other PAHs, the mutagenic and carcinogenic activities of DMBA are dependent on its conversion by the P-450/P_1-450 monooxygenase enzyme systems to chemically reactive electrophilic carbonium ions which will react covalently with critical cellular macromolecules especially DNA within the target tissue (*133-135*). This latter process is now considered to be the most probable initiation event in malignant transformation (*133-137*). Differences in susceptibility to DMBA have been observed between virgin and parous rats. This raises the possibility that hormones resulting from the

Table 4. Effect of degree of susceptibility.

	Mammary gland degree of susceptibility			
Parameters of susceptibility	High	Intermediate	Low	Reference
Morphological differentiation, cell kinetics	TEB>TD>AB>LOB	TEB<TD>AB>LOB	TD<AB<LOB	(*62, 99, 101, 102, 110*)
MI	7.03 ± 1.00	2.90 ± 2.30	0.09 ± 0.16	(*62, 83*)
DNA-LI	34.40 ± 7.60	14.80 ± 4.70	0.30 ± 0.50	(*83, 110, 113*)
Tc	9.93 ± 0.31	18.75 ± 0.99	49.63 ± 6.86	(*113*)
G.F.	0.55	0.19	0.0097	(*113*)
Lag phase in culture, hr	None	24	36	(*123, 124*)
No. of doublings in culture	3.04 ± 0.3	3.00 ± 0.14	1.50 ± 0.18	(*123, 124*)
^{3}H-DMBA uptake, grains/nucleus	6.80 ± 2.60	1.30 ± 0.80	0.70 ± 0.50	
DMBA-DNA binding, μmole/mole DNA-P	33	25	20	(*115*)
DNA repair				
UDS, % of cells	20.0 ± 2.8	37.3 ± 3.0	62.5 ± 3.0	(*128*)
Adduct removal at 24 hr, %	15	7	25	(*125*)
Carcinoma development	High	Intermediate	Low to none	(*83, 101*)
Benign lesion development	Intermediate	High	Low to none	(*83, 101*)

pregnancy and ensuing lactation could have modified the mammary epithelial cells in their ability to metabolize DMBA to its carcinogenic species, their ability to bind DMBA to DNA and to repair the resulting DMBA-induced DNA damage.

DMBA-DNA Binding by Rat Mammary Epithelium

After administration of DMBA to rats *in vivo* it binds to the DNA of mammary parenchymal cells with a ratio proportional to the rate of cell proliferation (*138-140*). It has been demonstrated that a correlation exists between the rats' age and level of DNA synthesis, which in turn is related to the amount of DMBA specifically bound to mammary gland DNA and the susceptibility of the gland to carcinogenesis (*138-140*). However, the observed differences in DMBA-DNA binding are due not only to age but also to the reproductive history of the rat, since there are differences in the amount of DMBA bound to the DNA of mammary epithelial cells derived from virgin and parous rats. It has been observed *in vitro* that at all time points, the highest level of DMBA binding occurs in young virgin cells, suggesting that they are more susceptible to the effects of increasing amounts of DMBA (*115*). The largest difference in terms of binding, however, are obtained in 24-hr cultures where binding in young virgin cells is 20-30% higher than in old virgin cells and 35-40% higher than parous cells over a dose range of 0.1-0.4 μg DMBA/mL. This correlates well with the fact that, at 24 hr, both old virgin and parous cells are still in a resting phase, whereas young virgin cells are already in their logarithmic phase of growth (*124*). This lower binding of DMBA during the lag phase of old virgin and parous cells *in vitro*, therefore, implies that both the small growth fraction and proliferative compartment in both groups of animals (*106*) are important factors in determining the eventual carcinogenic response by reducing the capacity of their cells to bind DMBA.

Therefore, young virgin rats, whose mammary glands contain numerous TEBs with a high proliferative activity show the highest uptake of ^{3}H-DMBA into mammary parenchymal cells *in vivo* as determined by autoradiographic techniques (Table 5). Uptake into TEB epithelium occurs selectively in the nucleus. More differentiated structures, such as ABs, which are the predominant structures in parous rat mammary gland, are characterized by having a low DNA synthetic activity and mitotic index, both of which are associated with a low DMBA uptake (*106*) and with a low DMBA-DNA binding *in vitro* (*115*). The significance of these results, therefore, lies in the relationship between the high nuclear uptake and the higher DNA synthetic activity in the TEBs (Table 6) and the fact that TEBs are the site of origin of mammary carcinomas (Table 6) (Fig. 3).

Identification of DMBA-DNA Adducts in Rat Mammary Gland

Identification of DMBA-DNA adducts in the mammary gland with the use of the Sephadex LH-20 chromatographic method developed by Baird and Brookes (*141*) reveals that the elution volumes of the DMBA-nucleic acid adduct peaks derived from young virgin, old virgin and parous cells are identical, suggesting that cells from the three groups of rats treated with DMBA *in vitro* generate similar adducts (*125*) (Fig. 11). These results are in agreement with those reported for the major DMBA-nucleic acid adducts generated in mouse skin (*142*), rodent embryo cells in culture (*143, 144*) and in human mammary epithelial cells (*145*) treated with DMBA. Although our results are consistent with the bay-region theory of polycyclic hydrocarbon carcinogenesis (*146*) and provide evidence that

Table 6. Correlation between the uptake of ^{3}H-DMBA and the DNA-LI in the different compartments of the mammary gland.[a]

	Structure	Grain/nucleus	DNA-LI
1	TEB	6.8 ± 2.6	34.4 ± 7.6
2	TD + ducts	1.3 ± 0.8	12.3 ± 5.8
3	AB	0.7 ± 0.5	7.9 ± 3.3

[a]Student's t-tests were done. The following comparisons were significantly different: grains/nucleus, 1 vs. 2, $p<0.01$, 1 vs. 3, $p<0.001$; DNA-LI, 1 vs. 2, $p<0.01$, 1 vs. 3, $p<0.001$, 2 vs. 3, $p<0.05$.

Table 5. Autoradiography of incorporation of ^{3}H-7,12-dimethylbenz(a)anthracene into the rat mammary gland.[a]

	Structure	No. of cells	Grains/ cell	Grains/ nucleus	Grains/ cytoplasm	Grains/ lumen
1	TEB	740	9.7 ± 2.9	6.8 ± 2.6	2.7 ± 0.9	0.97 ± 0.2
2	TD + Ducts	592	2.1 ± 1.4	1.3 ± 0.8	0.8 ± 0.6	0.98 ± 0.6
3	AB	345	1.0 ± 0.7	0.7 ± 0.5	0.5 ± 0.3	0.40 ± 0.3

[a]Student's *t*-tests were done. The following comparisons were significantly different: grains/cell, 1 vs. 2, $p<0.01$, 1 vs. 3, $p<0.001$; grains/nucleus, 1 vs. 2, $p<0.001$, 1 vs. 3, $p<0.001$; grains/cytoplasm, 1 vs. 2, $p<0.01$, 1 vs. 3, $p<0.01$. Labeled DMBA (^{3}H-DMBA, 1mCi) with 20 mg cold DMBA as a carrier was dissolved in 1 mL sesame oil and given IG to 55-day-old virgin rats. After 24 hr, the mammary glands were removed, fixed in Bouin's and processed for light microscopy. Paraffin sections were coated with NTB-2 Kodak emulsion and processed for autoradiography.

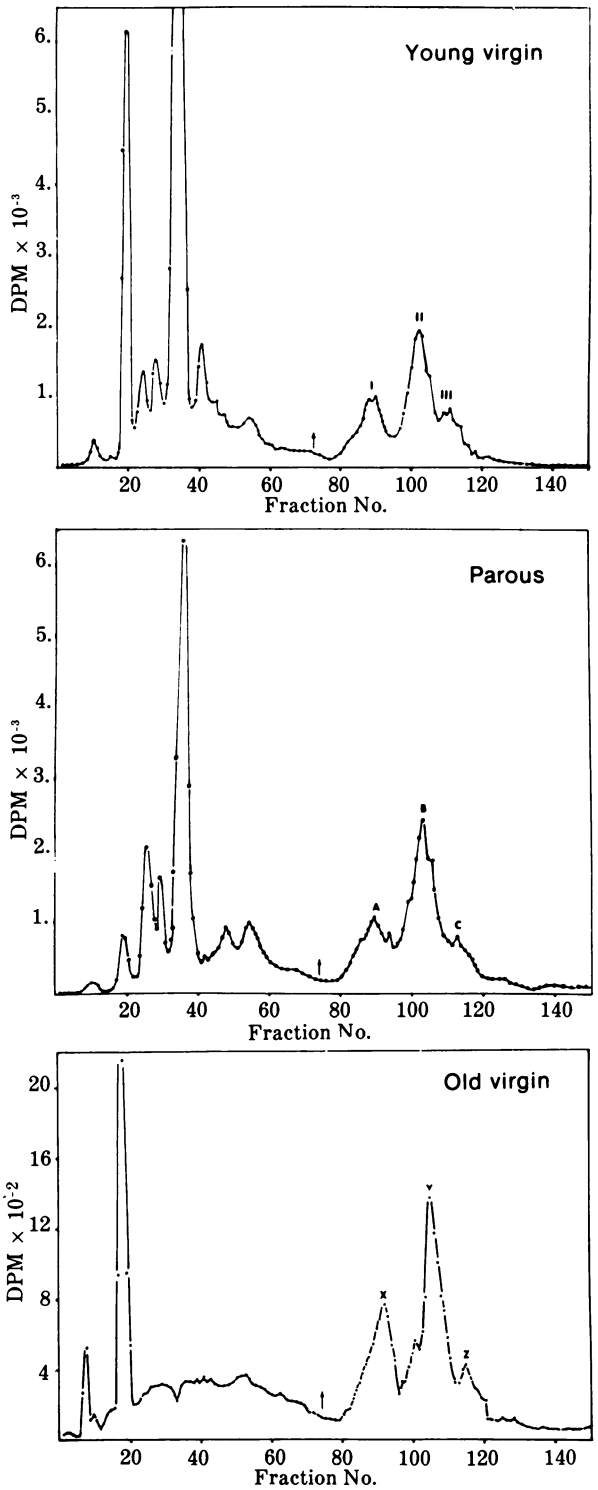

FIGURE 11. Elution profiles from Sephadex LH-20 columns of hydrolysates of DNA isolated from YV, P and OV rat mammary epithelial cells exposed to 0.5 μg ^{3}H-DMBA/mL for 24 hr; (Arrow) the position of elution of the added UV marker of 4-(*p*-nitrobenzyl)pyridine. Reprinted with permission of the editors from Tay and Russo (*125*).

DMBA-3,4-dihydrodiol-1,2-oxide is involved in the activation and binding of DMBA to DNA in mammary epithelial cells, the fact that adducts derived from virgin and parous rats, which are known to have different susceptibility to DMBA-induced mammary carcinogenesis, are identical, indicates that the susceptibility of the gland depends upon factors unrelated to the generation of different DNA adducts. It does not, however, rule out the possibility that the different susceptibility may be due to quantitative differences, since we have already demonstrated that the overall level of binding is different between virgin and parous rat mammary epithelial cells treated with DMBA *in vitro* (*115*).

DNA Repair by Rat Mammary Epithelium in Primary Culture

Carcinogen-induced DNA lesions are subject to repair by various mechanisms. Since repair of damaged DNA in mammalian cells appears to be an important process with regard to cell susceptibility and cell death, the ability of cells to repair damaged DNA will therefore determine the end biological result of the DNA-carcinogen interaction (*125, 147-153*). Since parous rats are refractory to the carcinogenic effect of DMBA, it has been postulated that their mammary gland epithelium must repair more efficiently the DMBA induced DNA damage.

The induction of unscheduled DNA synthesis (UDS) in 48-hr cultures of rat mammary epithelial cells following exposure to DMBA, measured by ^{3}H-thymidine incorporation into the nucleus of cells in the presence of hydroxyurea, indicates that induction of UDS is proportional to the dose of DMBA between 0.1 and 1.0 μg/mL. Induction of UDS is 2- to 4-fold higher in parous cells than in old virgin and young virgin cells, indicating a more efficient DNA repair process by cells of the parous rat mammary epithelium (Fig. 12).

Determination of DNA repair by measuring the efficiency of removal of DMBA adducts (*125*) following the protocol of Dipple and Hayes (*154*) reveals that in both young virgin and old virgin cells, the excision of adducts is very slow. For young virgin (YV) cells, the values show that at 5 and 18 hr, the quantitative measure of the excision of DMBA-DNA adducts obtained by calculating the time-dependent decrease in specific radioactivity of the less dense DNA peak following centrifugation on alkaline cesium chloride density gradients reveals that only 3% and 9% of the initial adducts are removed, increasing to only 24% at 48 hr. In old virgin cells, no loss of specific activity is detected within the first 18 hr, and the total loss of adducts after 48 hr is only 12% of the initial amount bound. Cells of parous rats, on the other hand, show an 8% loss of specific activity

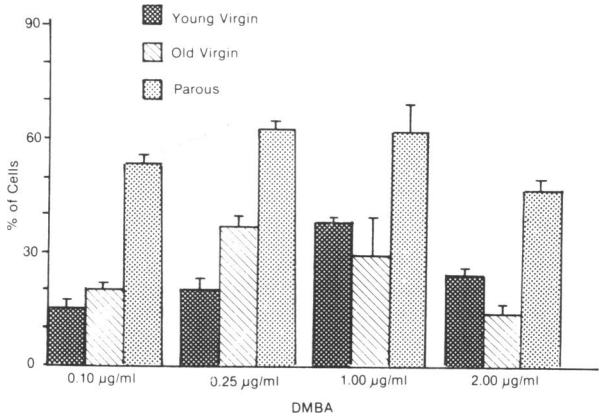

FIGURE 12. Induction of unscheduled DNA synthesis *in vitro* in YV, OV and P rat mammary epithelial cells exposed to DMBA for 24 hr.

within 5 hr. This loss steadily increases up to 38% by 48 hr. When the relationship between the amount of adducts excised at 24 and 48 hr is plotted against the initial extent of binding (Fig. 13), the results demonstrate that parous cells are capable of a greater and more rapid rate of adduct removal than either old virgin or young virgin cells. Thus, the low tumor incidence observed in parous rats may be due not only to a low DMBA-DNA binding, but also to more efficient DNA repair processes which may have arisen as the result of the cellular differentiation induced in the mammary epithelium by pregnancy and lactation.

Conclusions

The results described above indicate that susceptibility of the mammary gland to carcinogenesis is a composite of multifactorial aspects centered in the differentiation of the gland. The degree of differentiation of the gland as determined by morphological development, cell kinetics parameters and behavior in culture determines the affinity of the target to carcinogen-binding to DNA and the extent and proficiency in repair of damaged-DNA. Based upon these parameters it is possible to classify the susceptibility of the mammary gland to carcinogenesis as high, intermediate and low (Table 4). Thus measurement of one or more of these parameters can indicate the degree of susceptibility of a given mammary tissue to carcinogenesis. Preliminary results in our laboratory suggest that a correlation between human breast tissue and rat mammary gland findings is possible. (*155, 156*).

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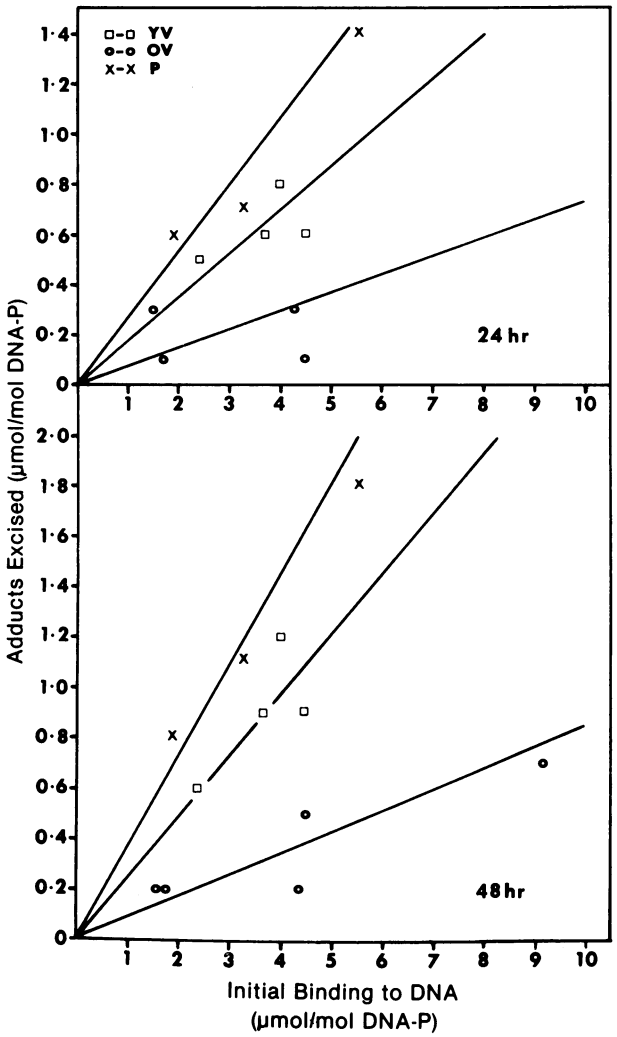

FIGURE 13. Relationship between the initial extent of binding of DMBA to DNA and the amounts of DNA adducts excised in 24 and 48 hr post-DMBA treatment. Confluent cell cultures from YV, OV and P rat mammary glands were treated with ^{3}H-DMBA (0.04-0.1 µg/mL, specific activity 1 Ci/mmole). Reprinted with permission of the editors from Tay and Russo (*125*).

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